PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : G01N 33/00 — A1

(11) International Publication Number: WO 00/45164

(43) International Publication Date: 3 August 2000 (03.08.00)

(21) International Application Number: PCT/US00/02122

(22) International Filing Date: 28 January 2000 (28.01.00)

(30) Priority Data:

60/118,013	29 January 1999 (29.01.99)	US
60/125,263	19 March 1999 (19.03.99)	US

(71) Applicant *(for all designated States except US)*: GENOMIC INSTRUMENTATION SERVICES, INC. [US/US]; 935 Washington Street, San Carlos, CA 94070 (US).

(72) Inventors; and

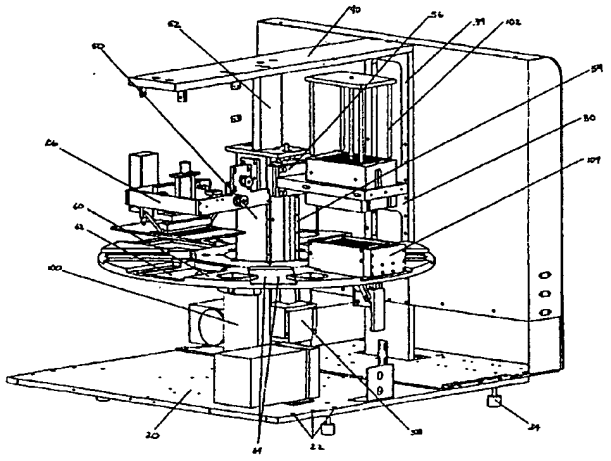
(75) Inventors/Applicants *(for US only)*: HUNICKE-SMITH, Scott, P. [US/US]; 58 Henderson Place, Menlo Park, CA 94025 (US). MEDEIROS, Omar [US/US]; 178 Permanente Way #3, Mountain View, CA 94040 (US). GUETTLER, Robert [US/US]; 2616 A 25th Street, San Francisco, CA 94110 (US). BUICE, Carl [US/US]; 2078 Montecito Avenue #3, Mountain View, CA 94043 (US). BERGSTEINSSON, Nancy [US/US]; 1086 Shotwell Street, San Francisco, CA 94110 (US). BATCHELDER, Keith [US/US]; 224 Robin Way, Menlo Park, CA 94025 (US).

(74) Agent: LUTHER, Barbara, J.; Barbara J. Luther, Chartered, 18124 Wedge Parkway, PMB 516, Reno, NV 89511-8134 (US).

(81) Designated States: AE, AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: ROBOTIC WORK STATION



(57) Abstract

An improved robotics workstation for biological and chemical processes is disclosed. In one embodiment the apparatus includes a base (20) on which the robotics workstation is arranged, at least two tools, each tool being arranged so that it may be used simultaneously with another tool for sample processing, a computer means (116) to run the workstation, a means to move (56) at least one tool vertically along a mounting column (52), a means to move (60) microwell plates (110) and at least one tool horizontally, whereby the vertically moved tool interacts with the horizontally moved microwell plate (110) or tool. Also disclosed are laboratory methods and customized workstations for their performance.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

Description

ROBOTIC WORK STATION

Field of Invention

The present invention relates to robotic workstations for chemical and biological synthesis, analysis and sample processing.

Background Art

Robotic workstations are becoming increasingly important, as there is an explosive increase in biological and chemical research. Modern research and clinical laboratory procedures include biological and chemical analysis of specimen substances that require extensive fluid manipulations. Standard fluid transfer and manipulative techniques include pipetting, diluting, dispensing, aspirating and plate washing. Most tools and robots are dedicated to the performance of one kind of operation. However, many areas of art are such that there are multiple manipulative steps, often of short duration, which must be performed in quick succession in order to achieve a given result. For example, chemical synthesis often requires multiple steps of heating, mixing, pipetting and centrifugation, each of which may be required within a very few minutes. It has simply been impractical to stop and change tools for each operation. The labor times necessary to recognize the need for the tool change and to implement the change often exceed the value of the robotic action by a particular tool. To be cost-effective, laboratory robots must provide high-throughput liquid handling of chemical and biological samples. Robots also must be easy to use and reliable. Scientists must be confident that they can design the experiment, prepare and start the instrument, and leave it unattended until various reactions are complete. Ideally, a robotic system should operate unattended for several hours or even overnight.

Many robotic workstations are being developed to address this need for high-throughput liquid handling of chemical and biological samples. One such workstation is disclosed in U.S. Patent No. 5,108,703, issued to Beckman Instruments in 1992. This patent discloses an automated, analytical chemistry processing system, or a multi-functional laboratory device, which has a movable interactive component for the controlled dispensing, aspirating and transferring of a liquid from a first microwell plate well or other fluid receptacle to a second microwell plate well or second fluid receptacle. It is capable of performing several functions; however, to do so, a technician must change out tools between every operation. The robot in this system has a single motor and a single movable and detachable tool. The system does not appear to be capable of performing two or more processes simultaneously.

Zymark produces a similar instrument, which it discloses in U.S. Patent No. 4,510,684, issued in April 1985. It discloses a robotic system for manipulating a series of discrete devices used in the field of analytical chemistry. It teaches the use of a robotic arm to open, contact and manipulate discrete laboratory devices in an emulation of manual methods on conventional laboratory instruments. However, the operations of the instrument are discrete and are not integrated into an intelligent coordinated system.

Several systems have been built which accommodate a variety of tools from different vendors and interface the tools by using robotic arms. One such system was created by the MIT Whitehead Genome Center and is called the Sequatron II. This system connects a wide variety of tools and instruments from different vendors to form an automated lab. However, it requires a great deal of reconfiguration and modification to the software, tools, and instruments for the system to be used for other applications.

High-throughput screening is developing in two important directions: ever larger machines and picoliter miniaturization. What is needed is a cost-effective robotic workstation on which a variety of tools and instruments can function simultaneously resulting in more efficient and effective high-throughput sample processing. Moreover, this robotic workstation should be flexible and capable of modification to meet the scientist's specific needs. Such a system makes the most effective and efficient use of expensive laboratory space.

Disclosure of Invention

It is an object of the instant invention to achieve reduced cost and increased flexibility in research by providing a robotic workstation that is customized at the factory rather than at the laboratory for a particular application. An improved robotic workstation for biological and chemical processes is disclosed. In one embodiment the apparatus includes a. a base on which the robotic workstation is arranged; b. at least two tools, each tool being arranged so that it may be used simultaneously with another tool for sample processing; c. a computer means to run the workstation; d. a means to move at least one tool vertically along a mounting column; e. a means to move microwell plates and at least one tool horizontally; whereby the vertically moved tool interacts with the horizontally moved microwell plate or tool. The tools of the workstation are selected from the following: 1-, 8-, 12-, 96-, 384-, and 1536-well pipetters and larger; thermocycler; arrayable centrifuge; pressure filtration station for filter bottom plates; spectrophotometer; fluorimeter; incubator for special gases; incubator for temperature control; gridder or microarrayer; shaker/ agitator for mixing or suspension; plaque or colony picking device; sample injector for off-instrument analysis; magnet for attraction of magnetic particles; electrophoresis unit; temperature control for deck plates; feedback sensors; bulk reagent dispenser; solid phase extractor; wash station; high performance liquid chromatography; gravimetric analysis apparatus; microplate cassette; and re-arrayer. Optionally, the workstation has a centrally located column to accommodate at least one centrally mounted tool block, to which the vertically moving tool is attached. The workstation's means to move the tool block vertically is a motor. The workstation's means to move the tool block is a lead screw and stepper motor. The workstation's means to move tools horizontally is a rotary deck powered by a motor that can be a D/C motor. The workstation preferably includes a server arm, which is programmed to move microwell plates on the deck or between adjacent decks.

An automated method of processing samples contained in microwell plates includes the following steps: a. providing an apparatus with a deck to accommodate at least one sample plate and at least one tool, a means for moving the deck, at least one mounting column accommodating at least one tool each, and each mounting column equipped with a means for moving tools, and a computer means to operate the apparatus; b. placing at least one sample plate on a deck; c. moving the deck to precisely position the sample plate and the tools, such tools being programmed to be capable of operating simultaneously to perform their respective functions; and d. removing the plates after the tools have performed respective functions on the samples contained therein.

An automated method of preparing cells for cell sorting includes the following steps: a. providing an apparatus with a deck to accommodate at least two sample plates, an array centrifuge, a pipetter, a wash station, and a bulk reagent dispenser; a means for moving the deck; at least one mounting column accommodating at least one tool each, each mounting column equipped with a means for moving the tools, and a computer means to operate the apparatus; b. placing at least one sample plate on a deck; c. moving the deck to precisely position the sample plate and the tools, such tools being programmed to be capable of operating simultaneously to perform their respective functions; and d. removing the plates after the tools have performed respective functions on the samples contained therein.

An automated method of performing solid phase extraction with a robotic work station has the following steps: a. providing a workstation with a deck to accommodate at least one sample plate, a microarray centrifuge, a server arm, microwell plates, and baskets for microwell plates and filter plates; a means for moving the deck; at least one mounting column on which are mounted a pipetter, a bulk reagent dispenser, and pressure station; at least one means for moving the column-mounted tools; and a computer with software modified to run the tools for the solid phase extraction; b. placing a sample plate with a plurality of wells containing a combination of growth media, bacteria and phage with cloned DNA onto the deck; c. aspirating the combination from the plurality of wells with the pipetter; d. pipetting the combination into an array centrifuge; e. centrifuging the combination to separate the cells and phage from a supernatant containing growth media; f. using the pipetter to remove the supernatants and placing them into a plurality of wells of a filter plate; g. using the reagent dispenser to add reagents to bind the phage to a plurality of wells of the filter plate; h. pressurizing the wells of the filter plate, thereby filtering the fluid and retaining the phage on the filter; i. adding reagents to lyse the phage and bind DNA to glass; j. pressurizing the wells of the filter plate to displace the fluid; k. washing the filters repeatedly with a solution to remove the salts; l. pressurizing the filters to dry them; m. placing a fresh sample plate under the filter plate; n. adding and incubating a reagent for resuspending the DNA from the filter plate; and o. pressurizing the filter plate to elute the incubated solutions into the underlying sample plate, thereby producing solutions of purified DNA suitable for sequencing.

Another embodiment is a robotic workstation for automated cell preparation for sorting cells. The workstation has a base on which the robotic workstation is arranged, an array centrifuge, a pipetter, a wash station, a bulk reagent dispenser, a server arm, each tool being arranged so that it may be used simultaneously with another tool for sample processing, a computer means to run the workstation; a means to move at least one tool vertically, a means to move microwell plates and at least one tool horizontally, whereby the vertically moved tool can interact with the horizontally moved microwell plate or tool.

Another embodiment is a robotic workstation for automated solid phase extraction. The workstation has a base on which the robotic workstation is arranged, an array centrifuge, a wash station, a bulk reagent dispenser, a server arm, each tool being arranged so that it may be used simultaneously with another tool for sample processing, a computer means to run the workstation, a means to move at least one tool vertically along a mounting column, a means to move microwell plates and at least one tool horizontally, whereby the vertically moved tool interacts with the horizontally moved microwell plate or tool.

Brief Description of the Drawings

Figure 1 is an overview of the robotic workstation with a pipetter, a wash station and a bulk reagent dispenser.

Figure 2 shows a cross section of the workstation.

Figure 3 shows a cross section of the workstation.

Figure 4 shows an overview of the robotic workstation with a pipetter, an array centrifuge, and a bulk reagent dispenser.

Mode(s) for carrying out the Invention

One of the best ways to achieve reduced cost and increased flexibility for the researcher is to provide a workstation that may be customized at the factory for a variety of particular applications. The key to the new workstation is a platform that accommodates a variety of tools or instruments appropriate to different applications and new tools and software adapted for each application. A scientist orders a "customized" workstation in which the platform is combined with the desired combination of tools, instruments, and software to meet the particular needs. In this way, costs are minimized because a significant fraction of the machine is unchanged from application to application, allowing for lower production costs of all machines. Each researcher receives a workstation suited to the desired applications, increasing flexibility. The combination of the majority of the mechanical, electrical, fluidic, and software components from the particular tools required for a given application benefits users in lower production costs and higher reliability than if they had purchased many separate custom instruments, often from different manufacturers and with conflicting software.

No other robotic workstation disclosed has the ability to combine a subset of tools and instruments so they may be used simultaneously on a platform to accomplish a task. The instrument disclosed in U.S. Patent No. 5,108,703 issued to Beckman Coulter has several tools that must be changed between every operation.

Zymark produces a robotic instrument, which it discloses in U.S. Patent No. 4,510,684 issued in April of 1985. It includes a robot with a tool assembly apparatus, which changes a single tool each time a different task is performed. However, it is not capable of simultaneously deploying two or more tools at different positions around the instrument.

Several systems incorporate a variety of tools from different vendors, such as Sequatron I and Sequatron II developed by the Whitehead Institute/ Center for Genome Research (see the Whitehead Institute web site) and interface between them using robotic arms. However, these systems require further integration in the laboratory, including custom programming and parts manufacturing. In contrast, the present invention is fully integrated, which allows for significant standardization among tools and applications and the cost savings that come with such standardization. The researcher benefits from dependability and lower installation costs when using a fully integrated system.

Disclosed herein is an automated workstation for chemical and biological synthesis and analysis. It provides great flexibility and functionality in a relatively small space. Modular tools are attached to a central column, which powered by a stepper motor, move the tools vertically to permit performance of a variety of functions on sample plates or other tools or instruments. The sample plates and other tools or instruments are precisely positioned on a rotary deck. The rotary deck is moved radially in the horizontal plane by a DC motor that has precise control to move tools attached thereto within relatively tight tolerances. A remote computer controls the workstation with software tailored to fit the scientist's particular needs. There is also a terminal, keyboard, and mouse with which the researcher can select the various processes and set parameters.

As shown in Figure 1, the workstation has a base 20, preferably made from light-weight non-corrosive material such as anodized aluminum, into which are machined a plurality of holes 22. These holes 22 permit the base 20 to be bolted to a work surface and accommodate leveling screws 24 to adjust for an angled surface. The holes also allow for the attachment of additional tools on the sides and/or corners of the apparatus. For example, a group of holes 22 can be used to bolt on a robotic arm.

The side support 30 is attached to the base 20. Shown in Figure 1 is one support 30. However, the supports can be any convenient number, which sufficiently stabilizes the entire apparatus but does not interfere with operator access to the deck and internal workings of the apparatus. The first end 32 of each support 30 is attached to the base. The second end 34 of the support 30 can optionally be attached to a cross bar 40 which also connects to the central support 50 via central column 52. This combination of base, supports, cross bar and contact of the central column with the base and the cross bar stabilizes the

apparatus. The central support 50 accommodates at least one track 54 in which modular tool mount block 56 moves vertically. The vertical movement is powered by a lead screw (not shown in Figure 1) and stepper motor 58. The modular tool block 56 carries a variety of tools, including a pipetter 102, a wash station 104, or a bulk reagent dispenser 106. Other optional tools are listed below.

Also shown in Figure 1 is the rotary deck 60 that revolves around the central column 50. It has one or more locations with specially shaped holes 62 which can accommodate microwell plates and deck locations with mounting holes 64. Additionally, the deck 60 has a variety of mounting locations to accommodate various tools or instruments. Horizontal movement of the deck 60 is powered by a deck motor 100, which is adjacent to the central support 50. The entire workstation is controlled by a remote computer (not shown) with software programmed to control the particular tools provided in the configuration of the workstation.

Figure 2 is a cross sectional view of the workstation, cutting through the center of the side supports 30 and the central column 52. The deck 60 is seen in cross section. The modular tool mount block 56 is shown in profile with its relation to lead screw 57 and stepper motor 58.

Figure 3 is a cross section of the workstation perpendicular to the plane shown in Figure 2. Slide ring 92 sits on the deck bearing plate 90 and contacts slide ring bearing 94. The deck motor 100 has a pinion gear (not shown) which actuates the slide ring 92 on the deck for precise motion to precisely align the deck 60 with a variety of tools and instruments. Also pictured is the electronics box 116, which contains all of the electronic circuitry to run the robotic workstation.

In Figure 4 one possible configuration of a set of tools and instruments is displayed. A pipetter 102 is attached to the central column 50 by a modular tool block 56. A bulk reagent dispenser 106 is also attached to the central column 50 by a modular tool block 56. A wash station 104 is nestled into one of the specially shaped holes 62. A microarray centrifuge 108 is attached to the rotary deck 60 at a deck location 64. The modular tool block 56 is powered by a lead screw and stepper motor 58 which moves the pipetter 102 in a precise vertical motion to interact with the microwell plates 110, wash station and microarray centrifuge 108 on the rotary deck 60. This configuration allows for a variety of centrifugation-based biological and chemical protocols to be run efficiently on one automated workstation. This is much more cost effective than purchasing each tool or instrument separately and manually or even robotically connecting them together. The workstation of Figure 4 can be used to process cells prior to cell sorting and in a variety of other procedures.

The robotic workstation can accommodate many tools, instruments, and applications. A brief list of tools includes, but is not limited to, the following:

1. 1-, 8-, 12-, 96-, 384-, and 1536-well pipetter (and larger) – A liquid handler for biological and chemical processing. A pipetter generally has a plurality of needles to aspirate from and deliver

samples into multiwell plates. The needles are anchored in a sample volume block and a needle guide assembly comprising a needle guide shaft, compression spring and needle guide plate, which are attached to the sample volume block and control the spacing of the needles. (GeneMachines, San Carlos, California).

2. Thermocycler - A machine that automates the alternating heat cycles for the process of polymerization chain reaction (PCR). (MJ Research, Watertown, MA; and Perkin Elmer, Foster City, CA)

3. Arrayable Centrifuge – An instrument used to separate heavier from lighter molecules and cellular components and structures. Recently, there has been an increasing demand for high-throughput assays in the field of biochemistry that has created a need for parallel processing and automation of many such protocols. In order to address those needs, GeneMachines™ offers a high-throughput automated centrifugation system in which samples are spun directly in contact with individual, miniature rotors rather than a sample holder. (GeneMachines, San Carlos, California).

4. Server Arm – A robotic arm utilized for moving sample plates to various destinations. An example of a server arm is the Garçon™: a modular, automated server arm designed to automatically deliver and retrieve microwell plates to and from other processing equipment (GeneMachines, San Carlos, CA).

5. Pressure Filtration Station - A positive-pressure device for filter plates. Filter-based preparation systems for nucleic acids have been shown to be useful in a variety of applications. These are commonly implemented using a centrifuge or vacuum filtration. Centrifugation gives excellent results but is classically difficult to automate. Vacuum methods are not capable of generating high enough pressures to filter more viscous solution, and they can cause cross-contamination through foaming or out-gassing of the filtrate. The GeneMachines Pressure Station eliminates these problems and gives you great control in a small package. (GeneMachines, San Carlos, California)

6. Spectrophotometer – An instrument that measures the concentration of a compound that has been dissolved in a solvent. The instrument shines a light through the solution, measures the fraction of the light that is absorbed by the solution, and calculates the concentration from that absorbance value. (Amersham Pharmacia Biotech- Buckinghamshire, United Kingdom; and Molecular Devices, Sunnyvale, CA)

7. Fluorimeter – An instrument used to measure fluorescence. It generates the wavelength of light required to excite the analyte of interest. The analyte of interest then emits a different wavelength that is measured by the fluorimeter. The emitted light is proportional to the concentration of the analyte being measured. (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom; and Dynex Laboratories, Farmington Hills, MI)

8. Incubator for Special Gases – Controlled environment for microplate incubation featuring control of temperature and atmosphere. Incubation occurs in user-defined atmospheres, for instance, enriched carbon dioxide or oxygen atmospheres, and with controlled humidity. This incubator supports cell growth in a wide variety of cell types. (Beckman Coulter, Palo Alto, CA)
9. Incubator for Temperature Control – An enclosure placed around an instrument for the regulation of temperature. (Fischer Scientific, Pittsburgh, PA and Thermolyne, Dubuque, Iowa).
10. Gridder or Microarrayer – An instrument used for arraying nucleic samples by eluting DNA samples onto a solid surface containing complementary DNA. The preferred microarrayer is a high performance, multi-axis microarrayer capable of arraying biological samples from standard 96- or 384-well microwell plates onto a variety of substrates, including glass slides and nylon membranes. The microarrayer incorporates features supporting routine arraying protocols, while providing a flexible, customized platform to meet specific research needs and accommodate future developments. (OmniGrid™, GeneMachines, San Carlos, CA).
11. Shaker/Agitator- An instrument that has positions for holding samples and mixes or suspends the samples by a rocking motion. The preferred shaker combines orbital shaking, oxygenation, and incubation in a high-capacity format for bacteria and phage growth. With these unique features, growth in standard 96-microwell plates meets most sequencing needs. (HiGro™, GeneMachines, San Carlos, CA and New Brunswick Scientific, Edison, NJ).
12. Plaque or colony picker - An instrument designed for the picking of isolated plaques and colonies. It has narrow tubes or pins that can pick up plaques and colonies from samples and deposit them to a designated position. A preferred high speed, high throughput picker of plaques and colonies accomplishes 2000 picks per hour and can pick colonies about 0.5 mm in diameter and about 0.5 mm apart. It can be coupled with a robotic arm to automate the entire process. Controlled by user-friendly software with a graphical user interface (GUI), it enables researchers to pick plaques and colonies automatically, saving time and money. (Gel-2-Well™, GeneMachines, San Carlos, CA; and BioRobotics, Woburn, MS).
13. Sample injector for off-instrument analysis – A tool that is capable of aspirating samples from one instrument and depositing them on another instrument for analysis. (Beckman Coulter, Palo Alto, CA)
14. Magnet for attraction of magnetic particles – Material which attracts magnetic particles and can help keep samples and instruments in their designated positions. GeneMachines offers this as part of its OmniGrid™ microarrayer. One way in which the samples are held in place is through magnetic force. (GeneMachines, San Carlos, CA).
15. Electrophoresis Unit – An instrument that automates the technique for separating molecules based on the differential movement of charged particles through a matrix when subjected to an electric

field. (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom; and BioRad, Hercules, CA)

16. Temperature control for deck plates – Individual deck positions may be equipped with temperature control capability. A microplate in a specific position is then cooled or heated in order to keep the samples at the predetermined temperature. This feature maintains plates and their contents at the required temperature prior to their use.
17. Feedback sensors – Sensors that send signals to a computer indicating the exact position of tools. GeneMachines utilizes these sensors in their RevPrep™ machine. (GeneMachines, San Carlos, CA)
18. Bulk reagent dispenser – A set of reservoirs from which controlled volumes of reagents are dispensed. This is offered as part of the RevPrep™ machine. (GeneMachines, San Carlos, CA)
19. Solid phase extractor – Solid-phase extraction (SPE) is an extraction method that uses a solid phase and a liquid phase to isolate one, or one type, of analyte from a solution. It is usually used to clean up a sample before using a chromatographic or other analytical method to quantitate the amount of analyte(s) in the sample (AutoGen, Framingham, MA).
20. Wash station – A liquid container for the cleaning of instruments. GeneMachines offers such an instrument on its RevPrep™ workstation. This wash station has a large well that contains cleaning solution. Other instruments are dipped into the well and cleaned off. The well is automatically drained and refilled before each new instrument is placed in the cleaning solution. (GeneMachines, San Carlos, California).
21. High Performance Liquid Chromatography – A process by which complex mixtures of different molecules may be separated from each other by subjecting the mixture to repeated partitioning. (Beckman Coulter, Palo Alto, CA and Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom and Perkin Elmer, Foster City, CA)
22. Gravimetric Analysis Apparatus – Yields of process steps could be monitored gravimetrically. Sample plates would be tared when empty, liquid analyte would be added to plate, dried, and then reweighed with dry analyte in plates. The mass of resulting analyte could be used to monitor process progress. This process could be useful for either a full plate of samples or for independent samples. (Metter, Toledo, Ohio has an instrument similar to the one disclosed).
23. Microplate Cassette – Storage hotels for sample plates. Microwell plates in a variety of densities have greatly improved the sample handling capabilities of high-throughput genomic research labs. Just as tubes were juggled by technicians 10 years ago, microwell plates are being juggled. Now scientists deal with a few dozen plates per day instead of a few dozen tubes. To facilitate another step in process simplification, GeneMachines provides Microwell Cassettes™ that organize microwell plate transfer, storage, and presentation (GeneMachines, San Carlos, CA).

24. Re-Arrayer – Re-arrayers efficiently rearrange or subtract identified libraries with maximum format flexibility. An available re-arrayer provides a 24-pin pneumatic head and autoloader, which enable full rearrangement of a library, selection of specific target wells for analysis, or replication. Libraries can be rearrayed on 96- and 384-well plates. (Flexys™, Genomic Solutions, Ann Arbor, MI).

In another embodiment, the robotic workstation stands alone without any side supports.

In another embodiment, the robotic workstation has tools or instruments attached to the base around the perimeter of the rotary deck to perform biological or chemical protocols.

In another embodiment, the workstation has one or more mounting columns supporting the rotary deck located on the periphery of the deck instead of centrally located.

In another embodiment, the workstation has an oblong deck that moves back and forth. Instead of a central column on which tools move vertically, the workstation has columns along one or both sides of the deck to accommodate tools and instruments. It is convenient to locate the tools along one side and at least one server arm on the opposite side.

In another embodiment, a server arm is provided in conjunction with the robotic workstation. The server arm contacts the microwell plates and moves them to various locations on the robotic workstation or to adjacent workstations. A chain of workstations can thus be linked with server arms, creating a fully automated chemical and biological laboratory for scientists.

In another embodiment, any machinable metals are utilized as material for the robotic workstation. Examples include stainless steel and aluminum.

In another embodiment, the computer controlling the robotic work station and its various tools and instruments is located on the rotary deck or on the base.

Optionally, the workstation has a heater positioned in the center or along a side or corner, which rapidly heats up the wells in the sample plate. Incubation may be performed at any temperature that facilitates the chemical reactions, typically between 4° and 40° C., more commonly between 15° and 40° C. Incubation periods are likewise selected for optimal binding but also minimized to facilitate rapid, high-throughput screening.

Another embodiment provides a method of preparing cells for cell sorting utilizing a customized robotic workstation.

Another embodiment provides a method of performing automated solid phase extractions utilizing a customized robotic workstation.

The following examples illustrate, but in no way are intended to limit, the present invention.

Example 1

One configuration of the robotic workstation is customized for the preparation of cells for cell sorting. Before cell sorting based on fluorescent-activated compounds, the desired cells need to be labeled with a fluorescent tag, such as a labeled antibody. The robotic configuration suitable for such cell preparation is illustrated in Figure 4 and combines the following tools and instruments: 1) an array centrifuge 108, 2) a pipetter 102, 3) a wash station 104, and 4) a bulk reagent dispenser 106. LabVIEW™ Software (LTR Publishing, Inc., Dallas, TX) is programmed to run this configuration of the robotic workstation.

A pipetter 102 is attached to the central column 50 by a modular tool block 56. Microwell plates 110 are nestled into deck locations with specially shaped holes 62 therein. Each well of a first microwell plate 110 contains a different cell sample or a control. A second microwell plate (not shown) contains antibodies in a plurality of its wells. The rotary deck 60 powered by the deck motor 100 revolves and carries the first microwell plate 110 containing the cells, until the plate is precisely aligned underneath the pipetter 102. The pipetter 102 is lowered by lead screw 57 attached to stepper motor 58 toward the rotary deck 60, and it aspirates the cells from the microwell plate 110. The pipetter 102 moves vertically to its original position while the second microwell plate containing the test antibodies is then moved on the rotary deck until it is precisely aligned beneath the pipetter 102. The pipetter 102 moves vertically towards the rotary deck 60 and dispenses the cells into the plurality of wells. The combinations of cells and antibodies then incubate in the second microwell plate.

A bulk reagent dispenser 106 is also attached to the central column 50 via a modular tool mount block 56. Vertical motion of the bulk reagent dispenser 106 is powered by a lead screw 57 and stepper motor 58. The microwell plate 110 containing the combination of antibodies and cells is moved horizontally on the rotary deck 60 until it is precisely aligned with the bulk reagent dispenser 106. The bulk reagent dispenser 106 moves vertically towards the rotary deck 60 and deposits wash solution into the plurality of wells of the microwell plate 110. Then the bulk reagent dispenser 106 moves back up to its original position, as the second microwell plate containing the combination of solutions is horizontally moved until it precisely aligns with the pipetter 102. The pipetter 102 moves vertically and aspirates the combined solutions.

An array centrifuge 108 with a plurality of wells is located on the rotary deck 60. The rotary deck 60, powered by the deck motor 100, revolves and places the array centrifuge 108 in a position precisely below the pipetter 102. The pipetter 102 moves down and dispenses the diluted, incubated combinations into a plurality of wells in the microarray centrifuge 108. While the pipetter moves up, the array centrifuge 108 is actuated, and the centrifugal force forms a supernatant and a cell pellet in each well. As the array centrifuge 108 comes to a complete stop, the pipetter 102 is then vertically moved to aspirate the supernatant from the plurality of wells of the microarray centrifuge. The pipetter 102 moves vertically back to its original position.

A wash station 104 occupies position 62 on the rotary deck 60. The rotary deck 60 is then moved in a horizontal direction until the wash station is precisely positioned below the pipetter 102. The pipetter 102 is then vertically moved towards the wash station 104 and dispenses the supernatant into it and washes the pipettes as well, by aspirating and dispensing repeatedly. The pipetter 102 is then vertically moved back to its original position.

The array centrifuge 108 is vertically moved until it is precisely aligned with the bulk reagent dispenser 106. The bulk reagent dispenser 106 moves vertically and deposits wash reagent into the plurality of wells of the array centrifuge 108. As the bulk reagent dispenser 106 moves vertically to its original position, the array centrifuge 106 resuspends the cell pellet by rapid changes in its rotational velocity. The array centrifuge 108 then centrifuges the mixture once again to separate a supernatant and a cell pellet. As the array centrifuge slows down, the deck moves to align the array centrifuge with the pipetter 102. The supernatant is removed by the pipetter 102 and dispensed into the wash station 104, and then the bulk reagent dispenser 106 adds more wash reagent into the array centrifuge 108. The addition of a wash, resuspension of the cell pellet, centrifugation and removal of a supernatant is repeated three times. After the completion of these steps, a clean cell pellet remains in the array centrifuge 108.

The array centrifuge 108 is horizontally moved and precisely aligned with the bulk reagent dispenser 106, and a labeling buffer is deposited into a plurality of wells of the array centrifuge 108. The array centrifuge 108 then resuspends the cell pellet into the labeling buffer by rapid changes in its rotational velocity. This combination of substances is incubated for one hour in the array centrifuge 108, or it can be removed and placed in a microwell plate 110 for the incubation period.

Once again, the bulk reagent dispenser 106 adds wash reagent to the incubated substance. This incubated substance is then centrifuged. The supernatant is removed by the pipetter 102 and disposed of in the wash station 104. More wash reagent is added to a plurality of the array centrifuge 108 wells; then there is resuspension and then centrifugation. The cycle of adding a wash reagent, resuspending, centrifuging and removing a supernatant is repeated twice.

The array centrifuge 108 is then realigned with the bulk reagent dispenser 106 and a re-suspension and fixing solution is added to the wells. This mixture is resuspended. This solution is then removed by the pipetter 102 and placed into an appropriate container for cell sorting.

The use of an integrated robotic workstation is much more cost effective and efficient for the preparation of cells for sorting than the use of separate tools or instruments for each step of the process.

Example 2

Another inventive configuration of tools and instruments on the robotic workstation can be used to perform automated solid phase extractions (SPE) in an efficient and cost-effective way. This configuration includes 1) a microarray centrifuge, 2) a bulk reagent dispenser, 3) a server arm, 4) microwell plates, and 5) baskets for microwell plates and filter plates. LabVIEW™ Software is programmed to run this

particular configuration of the robotic workstation. An example of an automated SPE using this machine is the isolation of DNA for sequencing from bacteriophage M13. By the time that the following procedure takes place, the bacteriophage have multiplied in the E. coli.

M13 and its host E. coli cells suspended in a growth media are contained in a plurality of wells of a microwell plate. The microwell plate is placed on the rotary deck of the robotic workstation by a robotic server arm. The rotary deck moves in a horizontal direction until the microwell plate is precisely aligned with the pipetter. The pipetter is vertically moved towards the deck where it aspirates the samples of growth media and cloned DNA from the microwell plate. The pipetter is then vertically moved to its original position.

An array centrifuge is located on the rotary deck of the robotic workstation. While the pipetter is moving vertically, the rotary deck moves in a horizontal direction until the array centrifuge is precisely aligned with the pipetter. The pipetter is vertically moved towards the array centrifuge and deposits the samples into a plurality of wells of the array centrifuge. The pipetter is then moved in a vertical direction back to its original location, while the array centrifuge is actuated, and the cells and supernatant containing M13 are separated. The pipetter is vertically moved towards the array centrifuge, the supernatant is aspirated, and the pipetter vertically moves back to its original position.

A filter plate with a plurality of wells is also located on the rotary deck. Underneath the filter plate is a basket that hangs from the rotary deck that is designed to accept a microwell plate. While the pipetter moves, the rotary deck moves horizontally until the filter plate is precisely aligned with the pipetter. The pipetter is vertically moved and deposits approximately 300µl of the M13 supernatant into a plurality of wells of the filter plate.

A bulk reagent dispenser is attached to the central column via a modular tool mount block. The vertical motion of the bulk reagent dispenser is powered by a lead screw and stepper motor. The filter plate is moved horizontally by the rotary deck until it is precisely aligned with the bulk reagent dispenser. The bulk reagent dispenser is vertically moved towards the filter plate and deposits 30 µL of 20% PEG 8000 in 2.5M NaCl into wells of the filter plate. The bulk reagent dispenser is moved back to its original position.

A pressure station is also attached to the central column via a modular tool mount, and its vertical motion is powered by a lead screw and stepper motor. While the reagent dispenser is moving vertically, the rotary deck horizontally moves the filter plate until it is precisely aligned with the pressure station. The pressure station is vertically moved towards the filter plate and exerts air pressure above the filter plate. The air pressure filters the reagent and supernatant and traps the M13 phage in the filter. The pressure station then vertically moves back to its original position, while the filter plate is moved horizontally to precisely align with the bulk reagent dispenser, where the filter is washed twice with 3M $NaClO_4$ in 70% EtOH to lyse phage and bind DNA to glass. Then the filter is washed six times with 70% EtOH to remove

salts. The bulk reagent dispenser then is returned to its original position. Simultaneously, the filter plate is moved horizontally to precisely align with the pressure station, which dries the filter plate with compressed air. The filter plate is then moved horizontally to precisely align with the bulk reagent dispenser. The bulk reagent dispenser adds 60 µl of 10 mM Tris-HCl, pH 8.5, containing 1 mM EDTA (TE) to each well and moves back to its original position. The mixture is incubated for two minutes and resuspends the clean DNA.

During incubation, the filter plate is then moved horizontally to precisely align with the pressure station. A robotic server arm then loads a microwell plate into the basket beneath the filter plate such that the wells of the microplate are precisely aligned with the wells of the microwell plate. The pressure station exerts air pressure and elutes the incubated solution into the microwell plate. The purified DNA in TE solution is collected in the wells of the microwell plate.

This configuration of tools on the robotic workstation can produce approximately 50µl of single stranded DNA per well at approximately 80 ng/µl.

Although the horizontal and vertical movements of the tools have for the most part been described sequentially, it should be understood that while one tools moves horizontally, another tool can be moving vertically. This simultaneous, computer-controlled precise movement of tools enables complex processes to be performed in minimal amounts of time.

It is to be understood that the above description is intended to be illustrative and not restrictive. Many embodiments will be apparent to those of skill in the art upon reviewing the above description. The scope of the invention should be determined not with reference to the above description but should instead be determined with reference to the appended claims, along with the full scope of equivalents to which such claims are entitled.

Industrial Applicability

The disclosed invention can be utilized to perform a great number of different operations in an automated, compact and precise system, which saves money and time. The same space can be adapted for the performance of many different assays and operations. Utilizing equipment that takes up a small amount of space and that can be used for multiple tasks creates a great savings in overhead costs. The automation of so many steps also eliminates operator error and leads to precise results. Moreover, unlike other systems that require expensive filter steps or time-consuming manual transfers to a centrifuge, the Robotic Workstation can isolate plasmid DNA significantly more quickly and cheaply because of the extent of automation and coordination. The inventive workstation is a cost effective apparatus for automating biological and chemical protocols.

Claims

We claim:

1. A robotic workstation for biological and chemical analyses, the apparatus comprising:

a. a base on which the robotic workstation is arranged;

b. at least two tools, each tool being arranged so that it may be used simultaneously with another tool for sample processing;

c. a computer means to run the workstation;

d. a means to move at least one tool vertically along at least one mounting column;

e. a means to move microwell plates and at least one tool horizontally;

whereby the vertically moved tool can interact with the horizontally moved microwell plate or tool.

2. The workstation of claim 1, wherein at least two tools are selected from the following list: 1-, 8-, 12-, 96-, 384-, and 1536-well pipetters and larger; thermocycler; arrayable centrifuge; pressure filtration station for filter bottom plates; spectrophotometer; fluorimeter; incubator for special gases; incubator for temperature control; gridder or microarrayer; shaker/ agitator for mixing or suspension; plaque or colony picking device; sample injector for off-instrument analysis; magnet for attraction of magnetic particles; electrophoresis unit; temperature control for deck plates; feedback sensors; bulk reagent dispenser; solid phase extractor; wash station; high performance liquid chromatography; gravimetric analysis apparatus; microplate cassette; and re-arrayer.

3. The workstation of Claim 1, wherein a single mounting column is centrally located and accommodates at least one tool block, to which the vertically moving tool is attached.

4. The workstation of Claim 1, wherein the means to move the tool block vertically is a motor.

5. The workstation of Claim 4, wherein the means to move the tool block comprises a lead screw and stepper motor.

6.. The workstation of Claim 1, wherein the means to move the horizontally moving tools is a rotary deck powered by a motor.

7. The workstation of Claim 6, wherein the means to move the rotary deck is a D/C motor.

8. The workstation of Claim 1, wherein one or more columns supporting the deck are located on the periphery of the deck instead of being centrally located.

9. The workstation of Claim 1, wherein the deck is oblong, with two long sides and two short sides, mounting columns for vertically moving tools are arranged along the long sides, and the two short sides of the deck moves horizontally.

10. The workstation of Claim 1, further comprising a server arm that is programmed to move microwell plates on the deck or between adjacent decks.

11. An automated method of processing samples contained in microwell plates, the method comprising the following steps of:

a. providing an apparatus with a deck to accommodate at least one sample plate and at least one tool, a means for moving the deck horizontally, at least one mounting column accommodating at least one tool each and each mounting column equipped with a means for moving the tools, and a computer means to operate the apparatus;

b. placing at least one sample plate on a deck;

c. moving the deck to precisely position the sample plate and the tools, such tools being programmed to be capable of operating simultaneously to perform their respective functions; and

d. removing the plates after the tools have performed respective functions on the samples contained therein.

12. An automated method of preparing cells for cell sorting, the method comprising the steps of:

a. providing an apparatus with a deck to accommodate at least two sample plates and an array centrifuge, wash station; a means for moving the deck; at least one mounting column accommodating a pipetter and a bulk reagent dispenser; a means for moving the pipetter and dispenser vertically on the column; and a computer with software modified to run the tools for the cell preparation protocol;

b. combining antibodies and a cell sample in a plurality of wells of a microwell plate for incubation to tag desired cells with antibody;

c. adding wash buffer to the combination of cells and antibodies;

d. centrifuging the combination in an array centrifuge, separating a supernatant and a cell pellet in a plurality of wells;

e. aspirating the supernatant out of the wells;

f. repeating steps c, d and e until the antibodies in solution are sufficiently separated from the antibody-tagged cells to produce a clean cell pellet;

g. adding labeling buffer to the remaining clean cell pellet;

h. resuspending the cell pellet and labeling buffer and incubating the combination;

i. repeating steps c, d and e until the label in solution is separated from the labeled cells; and

j. adding reagent to resuspend the cells,

whereby the cells are prepared to be sorted by their label or lack thereof.

13. An automated method of performing automated solid phase extraction with a robotic work station, the method comprising the steps of:

a. providing a workstation with a deck to accommodate at least one sample plate, a microarray centrifuge, a server arm, microwell plates, and baskets for microwell plates and filter plates; a means for moving the deck; at least one mounting column on which are mounted a pipetter, a bulk reagent dispenser and pressure station; at least one means for moving the column-mounted tools; and a computer with software modified to run the tools for the solid phase extraction;

b. placing a sample plate with a plurality of wells containing a combination of growth media, bacteria and phage with a cloned DNA onto the deck;

c. aspirating the combination from the plurality of wells with the pipetter;

d. pipetting the combination into an array centrifuge;

e. centrifuging the combination to separate the cells and phage from a supernatant containing growth media;

f. using the pipetter to remove the supernatants and placing them into a plurality of wells of the filter plate;

g. using the reagent dispenser to add reagents to bind the phage to a plurality of wells of the filter plate;

h. pressurizing the wells of the filter plate, thereby filtering the fluid and retaining the phage on the filter;

i. adding reagents to lyse the phage and bind DNA to glass;

j. pressurizing the wells of the filter plate to displace the fluid;

k. washing the filters repeatedly with a solution to remove the salts;

l. pressurizing the filters to dry them;

m. placing a fresh sample plate under the filter plate;

n. adding and incubating a reagent for resuspending the DNA from the filter plate; and

o. pressurizing the filter plate to elute the incubated solutions into the underlying sample plate, thereby producing solution of purified DNA suitable for sequencing.

14. A robotic workstation for automated cell preparation for cell sorting, comprising a base on which the robotic workstation is arranged, an array centrifuge, a pipetter, a wash station, a bulk reagent dispenser, a server arm, each tool being arranged so that it may be used simultaneously with another tool for sample processing, a computer means to run the workstation; a means to move at least one tool vertically, a means to move microwell plates and at least one tool horizontally, whereby the vertically moved tool can interact with the horizontally moved microwell plate or tool.

15. A robotic workstation for automated solid phase extraction, comprising a base on which the robotic workstation is arranged; tools comprising an array centrifuge, a wash station, a bulk reagent dispenser, a server arm, each tool being arranged so that it may be used simultaneously with at least one other tool for sample processing, a computer means to run the workstation; a means to move at least one tool vertically along a mounting column; a means to move microwell plates and at least one tool horizontally, whereby the vertically moved tool interacts with the horizontally moved microwell plate or tool.

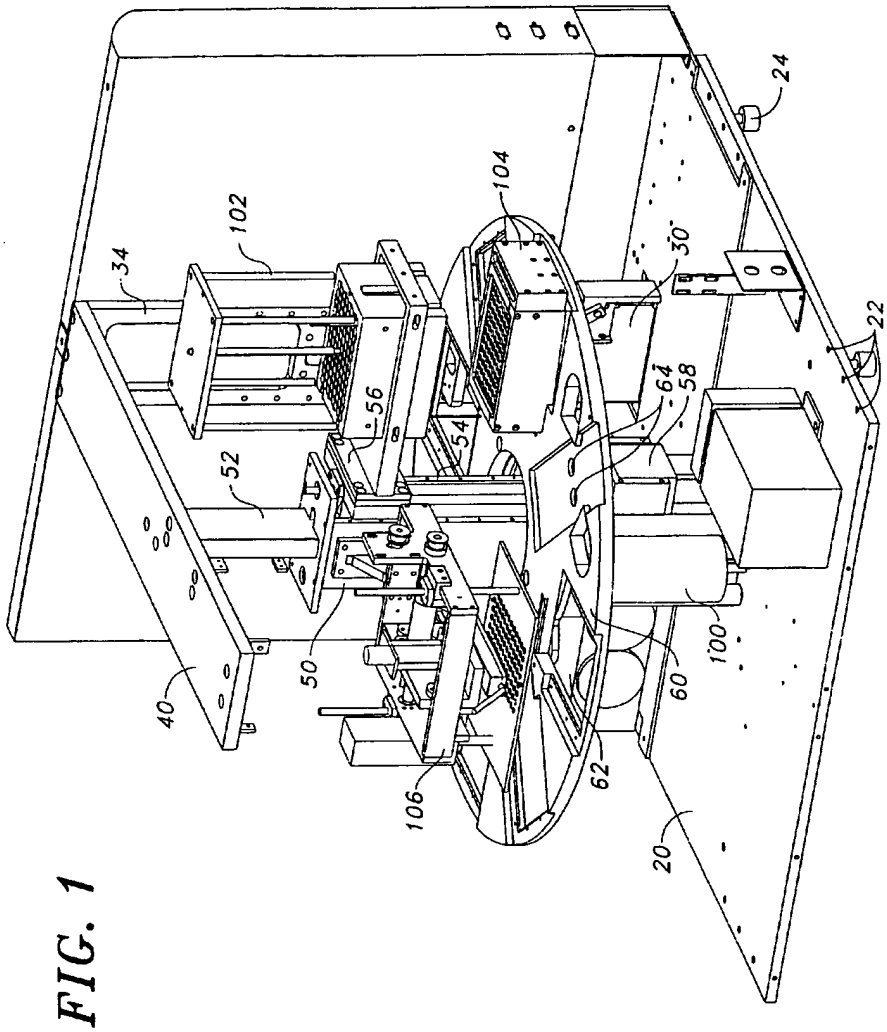


FIG. 1

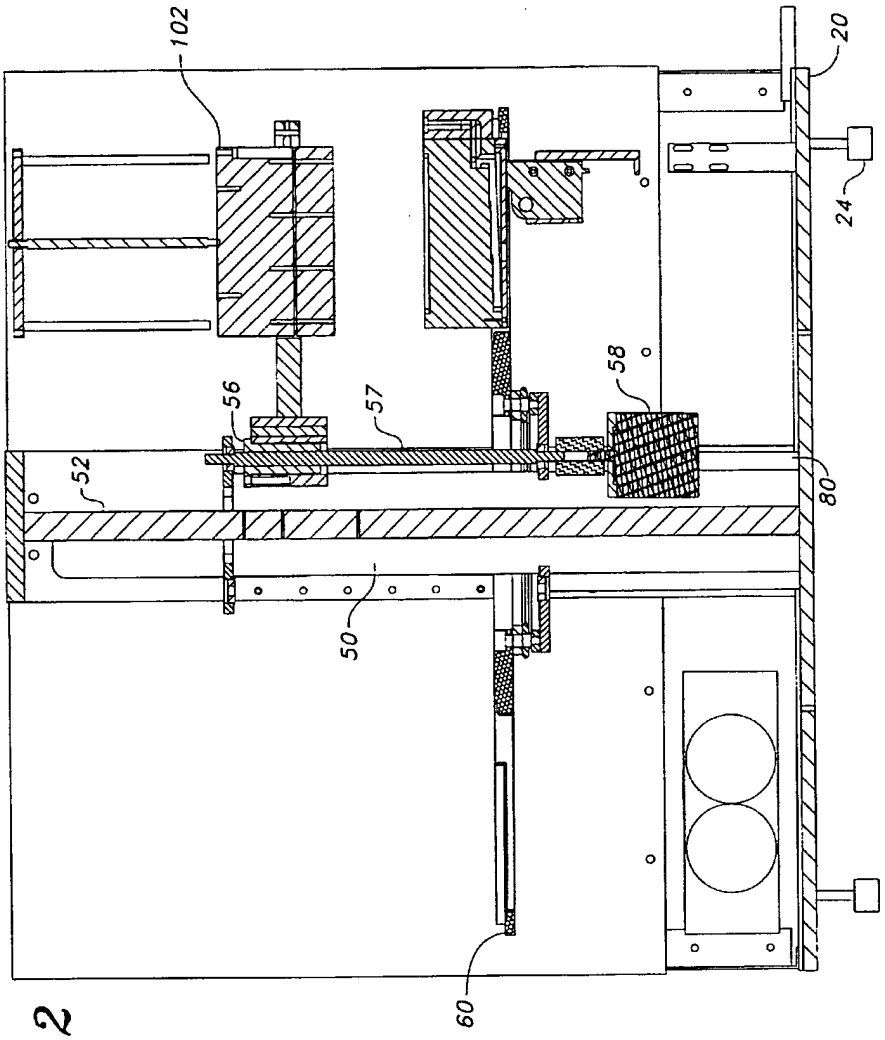


FIG. 2

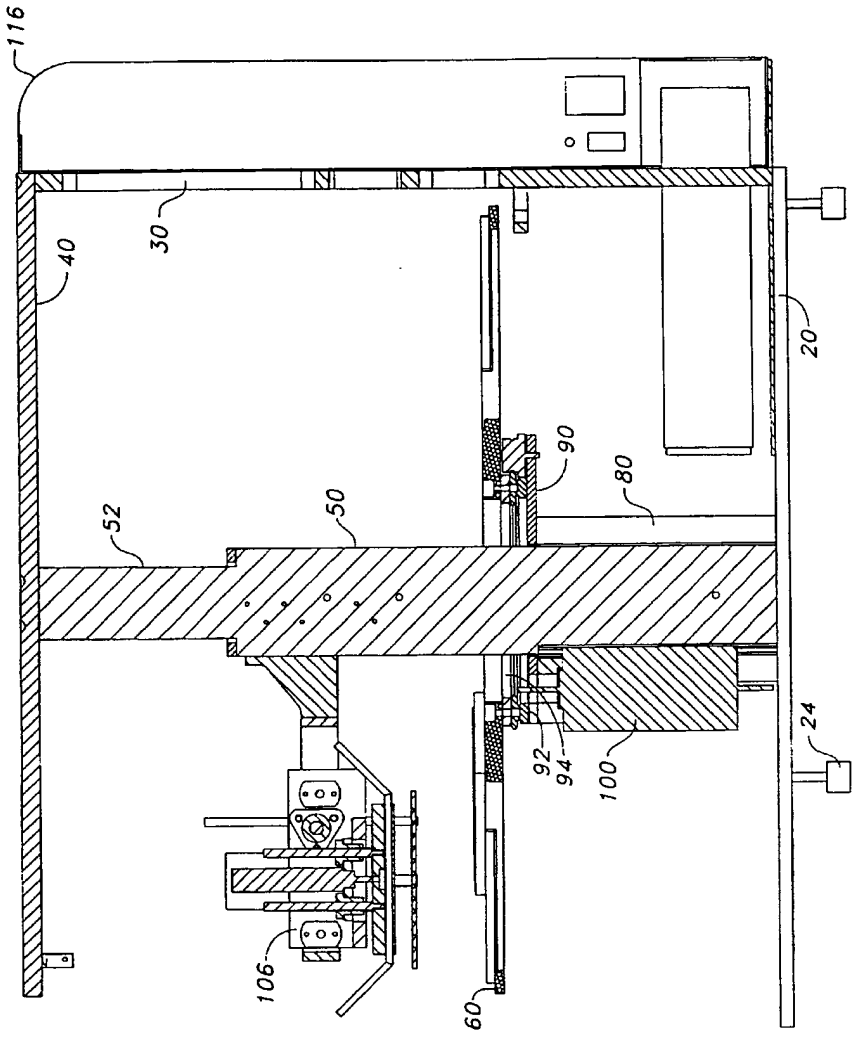


FIG. 3

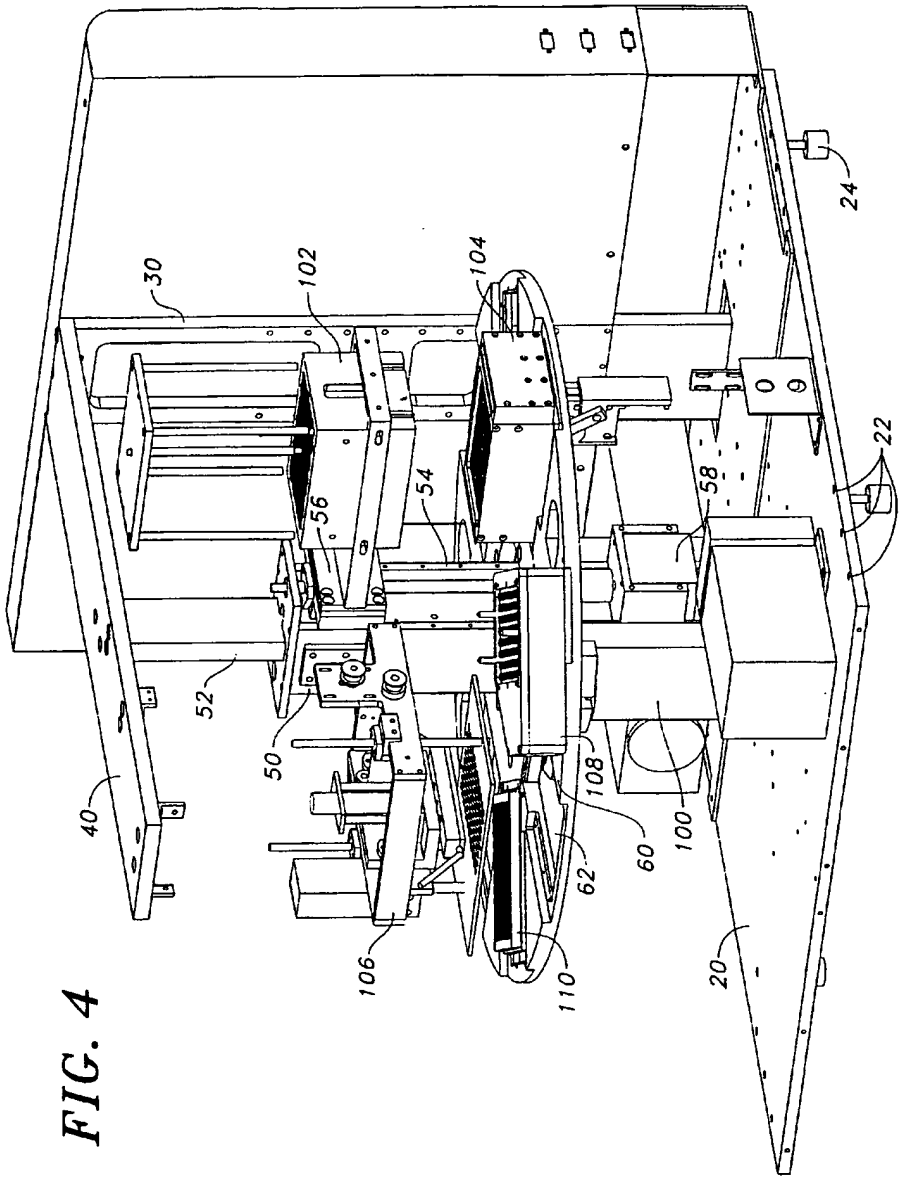


FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/02122

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :G01N 33/00

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/63, 64, 68.1, 72, 73, 100, 101; 436/43, 45, 47, 48, 49, 177, 178, 180

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BRS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,447,395 A (ENGLAR et al) 08 May 1984, entire document.	1-11, 14-15
Y	US 5,419,871 A (MUSZAK et al) 30 May 1995, entire document.	1, 4, 5
Y	US 5,102,623 A (YAMAMOTO et al) 07 April 1992, entire document.	1, 11
Y	US 5,459,073 A (RYAN) 17 October 1995, entire document.	12
Y,P	US 5,906,796 A (BLEVINS et al) 25 May 1999, entire document.	13
Y	US 5,603,160 A (LESSARD et al) 18 February 1997, entire document.	13

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

05 APRIL 2000

Date of mailing of the international search report

25 APR 2000

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

PATRICIA K. BEX

DEBORAH THOMAS
PARALEGAL SPECIALIST

Telephone No. (703) 308-0661

Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/02122

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,981,801 A (SUZUKI et al) 01 January 1991, entire document.	1-15
A	US 5,639,425 A (KOMIYAMA et al) 17 June 1997, entire document.	1-15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/02122

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

422/63, 64, 68.1, 72, 73, 100, 101; 436/43, 45, 47, 48, 49, 177, 178, 180